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PASTEURIZATION OF MILK ADVOCATED.

In 1907, when the Public Health Service made its study of the milk situation in its relation to the public health, the pasteurization of milk was urged as the only really dependable means of eliminating milk as a carrier of certain of the common communicable diseases, such as scarlet fever, diphtheria, septic sore throat, and typhoid fever. Following this a commission appointed to consider the milk question as it affected the city of Washington recommended municipal pasteurization. The pasteurization of milk has been advocated by many others. Recently at its meeting, October 15, 1917, the commission on milk standards, appointed by the New York City Milk Committee, adopted a resolution urging, for the protection of the health of the troops against diseases commonly carried by milk—

That all milk, including that which enters in the preparation of milk products, especially ice cream, be pasteurized and the efficiency of the process be controlled; that such milk be reduced to a proper temperature at the source of supply and kept at that temperature during transportation and until consumed; that the specifications for the purchase of milk be in conformity with the standards recommended by this commission.

THE BACTERIOLOGICAL EXAMINATION OF WATER.

COMPARATIVE STUDIES OF MEDIA USED.[1]

By H. E. HASSELTINE, Passed Assistant Surgeon, United States Public Health Service.

During the months of July and August, 1917, the writer, by direction of the Surgeon General, investigated a municipal water supply to ascertain whether the water complied with the Treasury Department [2] standard for water for use on interstate trains. As the third edition of "Standard methods for examination of water and sewage" (A. P. H. A., 1917) had appeared only a short time before, it was deemed advisable to follow its provisions.

[1] From the Hygienic Laboratory.

[2] Treasury Department Standard. Public Health Reports, vol. 29, Nov. 6, 1914, p. 2959.

It was suspected that the lactose broth made as directed in "Standard Methods" (1917) would not be reliable by reason of a probable breaking down of the lactose into simpler sugars, when sterilized at 15 pounds pressure for 15 minutes in the presence of organic matter. Consequently it was decided to run parallel tests of this new broth and the lactose broth prepared according to the method used at the Hygenic Laboratory for several years.

The ingredients used in preparation of the broth were Liebig's extract of meat, Witte's peptone, chemically pure lactose, and distilled water.

The broth was prepared according to the directions given in Standard Methods and the reaction made neutral to phenolphthalein. It was then divided into two equal portions. To the portion to be made into Standard Methods broth, 1 per cent of lactose was added and dissolved by shaking. The broth was then filled into Smith fermentation tubes and sterilized in the autoclave for 15 minutes after the pressure reached 15 pounds. This broth was in the autoclave about 1 hour, the time being divided as follows: 25 minutes to raise the pressure to 15 pounds, 15 minutes at that pressure, and about 20 minutes to allow the pressure to fall sufficiently to allow opening without blowing out or wetting the stoppers. The color of the medium treated in this manner was brown or yellowish-brown.

The portion of the original broth that was to be made into Hygienic Laboratory lactose broth was sterilized in bulk. To this a sufficient quantity of 20 per cent solution of lactose in distilled water, previously sterilized in an Arnold sterilizer for an hour and a half, was added to make 1 per cent lactose. This was then filled into sterile Smith fermentation tubes with reasonable precautions to prevent contamination in the filling process and the tubes were sterilized in the Arnold sterilizer for 30 minutes on one day only. This broth was usually a very pale yellow, nearly colorless.

The technique of the test was as follows: Samples of water were taken in a sterile bottle of 125 cubic centimeters capacity. After shaking the sample vigorously, five tubes of each kind of lactose broth were planted with 10 cubic centimeters, one with 1 cubic centimeter, and one with 0.1 cubic centimeter, using the same pipette for seeding both kinds of tubes. The planting of one kind of broth was never completed before the other was begun, it usually being the custom to plant two tubes of one kind and then two of the other until all were planted. The tubes were then incubated at 37° C. and the formation of gas was recorded at the end of 24 hours and again at the end of 48 hours.

From each tube showing gas formation at the end of 48 hours an Endo plate was made, which was incubated for 24 hours at 37° C. If the Endo plate showed typical colonies of *B. coli* (a red colony

with a greenish metallic luster) this was recorded as a positive test and further work deemed unnecessary. From all plates showing colonies that were not typical *B. coli* one or more colonies were fished to an agar slant, which was incubated 24 hours. The object of this deviation from the Standard Methods procedure was to insure sufficient growth to inoculate two fermentation tubes from one colony, or its descendants, as it was desired to transplant every colony fished into two kinds of lactose broth. It was found in some preliminary tests that it was not always possible to inoculate two fermentation tubes directly from a colony and to get growth in both. In the early tests smears were made to determine whether or not spore-bearing organisms were present, but after some experience it was found that the appearance of the growth was sufficient to determine this point in practically every case. At least one colony of each type present, other than spore-bearing organisms, was fished from each plate if the plate did not show typical *B. coli* colonies.

From these agar slants a fermentation tube of each kind of lactose broth was inoculated and incubated for 48 hours. The Endo plates were reexamined at the end of 48 hours at 37° C., but in no case was there any appearance of typical *B. coli* colonies as a result of this additional incubation. They were then left in the dark for 48 to 72 hours at room temperature and reexamined. A few plates showed a colonlike colony, but the one that was studied further proved to be not *B. coli*.

With the exception of the first 11 samples, further intensive work was done to determine if any *B. coli* were missed. The following procedure was carried out: From the original presumptive tubes showing gas formation, regardless of the amount of gas, a transfer was made directly to a second fermentation tube of lactose broth which was then incubated for 48 hours. If *B. coli* was not found in the first Endo plate or confirmation test and gas appeared in this second presumptive tube, a third fermentation tube and an Endo plate were inoculated from the second presumptive tube. If this Endo plate showed typical colonies of *B. coli* it was called positive. If colonies were only suspicious, confirmatory tests were tried. After 48 hours an Endo plate was made from the third presumptive tube, if gas was present, and this plate carried through the same procedure.

Comparison of Lactose Broth Made According to the Standard Methods Procedure and that Made by the Hygienic Laboratory Method.

In the work done on comparing the "Standard Methods, 1917," and Hygienic Laboratory lactose broth, 32 samples of water were examined, which may be divided into four classes: (1) raw water, (2) filtered water, (3) chlorinated filtered water taken at filter plant, and (4) chlorinated filtered water from taps in the city. The results of the presumptive test are set forth in Table 1.

TABLE 1.

Class of sample.	Number of sample.	Gas in 24 hours. 10 cc. Tubes planted.	10 cc. H. L. broth.	10 cc. S. M. broth.	1 cc. Tubes planted.	1 cc. H. L. broth.	1 cc. S. M. broth.	0.1 cc. Tubes planted.	0.1 cc. H. L. broth.	0.1 cc. S. M. broth.	Gas in 48 hours. 10 cc. H. L. broth.	10 cc. S. M. broth.	1 cc. H. L. broth.	1 cc. S. M. broth.	0.1 cc. H. L. broth.	0.1 cc. S. M. broth.	*B. coli* proven in— 10 cc. H. L. broth.	10 cc. S. M. broth.	1 cc. H. L. broth.	1 cc. S. M. broth.	0.1 cc. H. L. broth.	0.1 cc. S. M. broth.
Raw	1	5	5	5	2	2	2	2	1	1	5	5	2	2	1	2	5	5	2	2	1	1
	17	1	1	1	5	1	1	2	0	0	1	1	4	5	1	2	1	1	3	1	1	0
	25	1	0	1	5	0	1	2	0	0	1	1	5	5	1	2	1	1	1	1	0	0
Total	...	7	6	7	12	3	4	6	1	1	7	7	11	12	3	6	7	7	6	4	2	1
Filtered	3	5	5	2	1	0	0	1	0	0	5	5	2	2	2	0	3	2	0	0	0	0
	18	5	0	2	1	0	0	1	0	0	4	5	1	1	1	1	2	2	0	0	0	0
	26	5	0	2	1	0	0	1	0	0	5	5	1	1	1	0	5	3	0	0	0	0
Total	...	15	5	6	3	0	0	3	0	0	14	15	4	4	4	1	10	7	0	0	0	0
Chlorinated	2	5	0	0	1	0	0	1	0	0	3	5	0	0	0	0	0	0	0	0	0	0
	19	5	0	0	1	0	0	1	0	0	3	5	0	0	0	0	0	0	0	0	0	0
	27	5	0	1	1	0	0	1	0	0	4	5	0	0	0	1	0	1	0	0	0	0
Total	...	15	0	1	3	0	0	3	0	0	10	15	0	0	0	1	0	1	0	0	0	0
Tap	4	5	0	0	1	0	0	1	0	0	1	5	0	0	0	0	0	1	0	0	0	0
	5	5	1	0	1	0	0	1	0	0	4	4	0	0	0	0	0	1	0	0	0	0
	6	5	0	0	1	0	0	1	0	0	1	5	1	0	0	0	0	0	0	0	0	0
	7	5	0	0	1	0	0	1	0	0	2	5	0	0	0	0	0	1	0	0	0	0
	8	5	0	0	1	0	0	1	0	0	4	5	0	0	0	0	2	0	0	0	0	0
	9	5	0	2	1	0	0	1	0	0	2	5	0	0	0	1	0	0	0	0	0	0
	10	5	0	0	1	0	0	1	0	0	0	5	0	1	0	0	0	0	0	0	0	0
	11	5	0	0	1	0	0	1	0	0	0	5	0	0	0	0	0	0	0	0	0	0
	12	5	0	0	1	0	0	1	0	0	0	5	0	0	0	0	0	0	0	0	0	0
	13	5	0	0	1	0	0	1	0	0	4	5	0	1	0	0	0	0	0	0	0	0
	14	5	0	0	1	0	0	1	0	0	4	5	0	1	0	1	1	0	0	0	0	0
	15	5	0	0	1	0	0	1	0	0	2	4	1	0	0	0	0	0	0	0	0	0
	16	5	0	0	1	0	0	1	0	0	1	2	0	0	0	0	0	0	0	0	0	0
	20	5	0	0	1	0	0	1	0	0	2	4	0	1	0	0	0	0	0	0	0	0
	21	5	0	0	1	0	0	1	0	0	4	5	0	0	0	0	0	0	0	0	0	0
	22	5	0	2	1	0	0	1	0	0	4	5	0	0	0	0	3	5	0	0	0	0
	23	5	0	0	1	0	0	1	0	0	2	5	0	0	0	0	0	0	0	0	0	0
	24	5	0	0	1	0	0	1	0	0	1	4	0	1	0	0	0	0	0	0	0	0
	28	5	0	0	1	0	0	1	0	0	2	5	0	1	0	0	1	0	0	1	0	0
	29	5	0	0	1	0	0	1	0	0	2	2	0	1	0	0	0	0	0	0	0	0
	30	5	0	1	1	0	0	1	0	0	2	4	0	0	0	0	0	0	0	1	0	0
	31	5	0	0	1	0	0	1	0	0	1	5	0	0	0	0	0	0	0	0	0	0
	32	5	0	0	1	0	0	1	0	0	3	5	0	0	0	0	1	0	0	0	0	0
Total	...	115	1	5	23	0	0	23	0	0	48	104	2	7	0	2	8	8	0	2	0	0

TABLE 2.—*Summary of totals of Table 1.*

	No. of samples.	Gas in 24 hours. 10 cc. Pl.	10 cc. H. L.	10 cc. S. M.	1 cc. Pl.	1 cc. H. L.	1 cc. S. M.	0.1 cc. Pl.	0.1 cc. H. L.	0.1 cc. S. M.	Gas in 48 hours. 10 cc. H. L.	10 cc. S. M.	1 cc. H. L.	1 cc. S. M.	0.1 cc. H. L.	0.1 cc. S. M.	*B. coli* proven. 10 cc. H. L.	10 cc. S. M.	1 cc. H. L.	1 cc. S. M.	0.1 cc. H. L.	0.1 cc. S. M.
Raw	3	7	6	7	12	3	4	6	1	1	7	7	11	12	3	6	7	7	6	4	2	1
Filtered	3	15	5	6	3	0	0	3	0	0	14	15	4	4	4	1	10	7	0	0	0	0
Chlorinated	3	15	0	1	3	0	0	3	0	0	10	15	0	0	0	1	0	1	0	0	0	0
Tap	23	115	1	5	23	0	0	23	0	0	48	104	2	7	0	2	8	8	0	2	0	0
Grand total	32	152	12	19	41	3	4	35	1	1	79	141	17	23	7	10	25	23	6	6	2	1

From 228 tubes of H. L. lactose broth planted, *B. coli* was confirmed in 33.

From 228 tubes of S. M. lactose broth planted, *B. coli* was confirmed in 30.

Of these 63 confirmed *B. coli*, 52 were proved by Endo plates alone. Twenty-six of these plates were seeded from tubes of H. L. broth and the remaining 26 from tubes of S. M. broth. Seven tubes of H. L. broth and four of S. M. broth required a second lactose broth tube, because typical colonies were not found on the Endo plate.

From these figures it will be seen that owing to the greater incidence of gas formation in the S. M. broth 71 more tubes had to be carried through the confirmation test than when the H. L. broth was used. Notwithstanding the increased amount of work thus necessitated, the number of *B. coli* confirmed by plate or further fermentation test was slightly less than that obtained from the tubes of H. L. broth. This difference, however, is too slight to receive consideration. In other words, the lactose broth sterilized at 15 pounds pressure for 15 minutes required 36 (dividing a plate between two tubes) more Endo plates and 142 more tubes of lactose broth to find the same number of *B. coli* that were found when lactose broth sterilized at 100° C. for 30 minutes was used.

Comparison of Standard Methods Confirmed Test with that Required by the Treasury Department.

In the confirmation test another departure was made from the Standard Methods procedure, in that at least 10 per cent of gas (Treasury Department standard) in Hygienic Laboratory lactose broth was required in order to record it as a positive result. The Standard Methods procedure classifies as a member of the *B. coli* group any aerobic nonspore-forming organism that, fished from Endo plates seeded from the original fermentation tube to a second fermentation tube of lactose broth prepared as directed in Standard Methods, shows gas formation in the second fermentation tube within 48 hours. Using the broth, prepared in accordance with Standard Methods, the writer was able to use a pure culture of *B. proteus* and obtain results that would necessitate classifying it as *B. coli* by following the procedure advised in Standard Methods. Of course the colonies of this organism were far from typical on Endo plates.

The table following shows the results of the confirmation tests in the two kinds of lactose broth.

TABLE 3.

Number of plates fished from—	Number of positive, accepting any amount of gas as + result.		Number of positive, accepting 10 per cent of gas as + result.	
	H. L. broth.	S. M. broth.	H. L. broth.	S. M. broth.
201 (68 seeded from H. L. tubes, 133 seeded from S. M. tubes)...	20	51	10	25
Excess of S. M. + over H. L.....	.....	31	.....	15

From Table 3 it appears that if we accept gas formation, regardless of the amount, in the final lactose tube as indicative of *B. coli* we will practically double our positive findings. If we use lactose broth which is sterilized at 120° C. for 15 minutes we will more than double the positive findings obtained with lactose broth made according to the Hygienic Laboratory procedure. In other words, this indicates that the new Standard Methods procedure may give approximately four to five times more positive *B. coli* results than the Treasury Department procedure when the broth is made according to the Hygienic Laboratory method. This is based on the assumption that a colony is fished from every Endo plate showing aerobic colonies of nonspore-forming organisms.

Intensive work on the tubes which gave negative confirmation tests resulted in isolating *B. coli* from four samples which would have been reported negative by either procedure. Of this number all four showed no gas in the lactose fermentation tube of the confirmation test when Hygienic Laboratory broth was used, and three out of four showed no gas in the corresponding tubes of Standard Methods broth. One showed a bubble in Standard Methods broth. To the writer it appears that less than 10 per cent of gas in the final lactose tube of the confirmation test, can be disregarded without any appreciable danger.

Comparison of Endo Medium Made According to "Standard Methods" and Hygienic Laboratory Procedures.

After carefully reading the Standard Methods requirements for Endo medium it was suspected that Endo medium prepared according to the Hygienic Laboratory method, and the same medium prepared according to the Standard Methods procedure, would show different results if submitted to comparative tests.

The Hygienic Laboratory-Endo medium consists of a 3 per cent agar which is titrated and corrected to +0.5 to phenolphthalein, to which is added 3.7 cubic centimeters of a 10 per cent solution of anhydrous sodium carbonate. For convenience it is flasked, sterilized, and stored in 200 cubic centimeter quantities. When ready

to use the following ingredients are added to 200 cubic centimeters of agar as follows:

(*a*) Dissolve 2 grams C. P. lactose in 25 to 30 cubic centimeters of distilled water, with the aid of gentle heat.

(*b*) Dissolve 0.5 gram of anhydrous sodium sulphite in 10 to 15 cubic centimeters of distilled water.

(*c*) To the sulphite solution add 1 cubic centimeter of saturated solution of basic fuchsin in 95 per cent alcohol.

Add the fuchsin-sulphite solution to the lactose solution, and then add the whole to the agar. Pour plates at once and, after hardening, dry for 15 minutes in the incubator.

The Standard Methods Endo medium consists of a 3 per cent agar made neutral to phenolphthalein, flasked, sterilized, and stored in convenient quantities. When ready to use, to 200 cubic centimeters of agar there are added 2 grams of C. P. lactose and the agar is then melted in the Arnold sterilizer. A 10 per cent solution of anhydrous sodium sulphite is prepared and to 10 cubic centimeters of this solution 2 cubic centimeters of a 10 per cent solution of basic fuchsin in 95 per cent alcohol are added, and this solution is heated for a few minutes. To the 200 cubic centimeters of melted lactose agar is then added 1 cubic centimeter of the fuchsin-sulphite solution. Plates are poured and, when hardened, placed in the incubator for drying.

At first it was thought that the reaction of the agar might account for differences; but titration showed both agars to react the same (+0.8), using phenolphthalein as an indicator. The chief difference in the two media lies in the proportion of fuchsin and of sulphite used. The Standard Methods Endo contains but about one-fifth as much of these ingredients as does the Endo which has been found most useful at the Hygienic Laboratory.

The strength used by the Standard Methods was recommended by Kendall and Walker [1] for use in isolating *B. dysenteriae* from stools, a procedure in which a medium that promoted the formation of colorless colonies was desired. In the use of Endo in water examination colored colonies are sought. It therefore seems rational to use enough of the ingredients that promote color formation to give a reasonable coloration.

In the examination of water the use of an Endo medium that gives a typical colon colony enables the examiner to dispense with a vast amount of work, since the partially confirmed test, when colonies are typical, is almost as certain as the completely confirmed test. Of course atypical colonies must be confirmed, but if the number of typical colonies can be increased the work of confirmation, if required, will be reduced.

[1] Kendall and Walker. Jour. Med. Research, vol. 23, 1910, p. 481.

In the comparison of these two media a plate of each (using half a plate for each tube), was seeded from every fermentation tube showing gas. At the end of 24 hours' incubation the plates were examined. An additional 24 hours' incubation did not develop any typical colonies on plates that did not show typical colonies at the end of 24 hours.

One hundred twenty-nine plates of each kind of Endo medium were inoculated from a like number of fermentation tubes showing gas. The comparative results are shown in the following table:

TABLE 4.

Endo medium.	Number plates made on each medium.	Number showing typical B. coli colonies.	Number showing atypical aerobic colonies.	Number showing no aerobic colonies.
Hygienic laboratory	129	27	97	5
Standard methods	129	5	120	4

From the tubes showing atypical colonies, confirmatory tests and routine study demonstrated *B. coli* in 9. Three were from H. L. Endo, 3 from S. M. Endo, and 3 from both.

When typical *B. coli* colonies were found on either kind of medium, the sample was recorded as positive and the corresponding negative, or doubtful, plates of the other medium were not carried further. In no instance did the Standard Methods medium show typical *B. coli* colonies when the Hygienic Laboratory plate seeded from the same tube showed atypical colonies.

During the progress of the work, it was noted that the spore-bearing aerobes were much more restrained on the Hygienic Laboratory Endo medium than on the Standard Methods medium.

It is assumed that if the two media were equally good for the demonstration of *B. coli* an equal number of plates should show typical colonies. But 22 plates of Standard Methods Endo medium failed to show typical *B. coli* colonies while the corresponding Hygienic Laboratory plates showed typical colonies. In view of the number of *B. coli* subsequently demonstrated from plates showing atypical results, it would appear that the H. L. Endo medium shows typical colonies in 75 per cent of the tubes in which the *B. coli* is present and the S. M. Endo medium in 14 per cent. Since *B. coli* is sought as the index of contamination it would appear to be good policy in the examination of water samples to use an Endo medium designed to demonstrate *B. coli* rather than one modified to demonstrate some other intestinal organism.

Conclusion.

The results of this work indicate that if the new Standard Methods (1917) be adhered to, in the bacteriological examination of water, time, labor, and material will be unnecessarily expended and misleading results may be obtained.

THE SIMULATION OF DISEASE.

DRUGS, CHEMICALS, AND SEPTIC MATERIALS USED THEREFOR.

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This paper is not intended to be an exposé of all of the various methods of effecting simulation of disease, but is restricted to that phase of the subject involving the use of drugs, chemicals, and septic materials. This phase is of special interest at the present time, as it comprises the means most frequently employed by unscrupulous individuals in attempts to evade military duty. For the purpose of enhancing the value of the paper as a source of reference to the medical examiner, the substances enumerated therein are grouped under the diseases the diagnostic signs of which their use is intended to simulate. For the same reason, brief outlines of the methods recommended for the detection of these frauds are also included, where specific information of this kind has been available.

Substances used in the Simulation of Diseases of the Skin and Subcutaneous Tissue.

ERYTHEMA:[1] Certain nettles, poison ivy, squills, and some plants of the families *Euphorbiaceæ* and *Ranunculaceæ*. These are applied to the skin with friction.

ECZEMA: After abrading the skin, by scraping with a sharp-edged instrument or rubbing with some rough material, one, or more, of the following is applied: Croton oil, sulphur, acid substances, oil of cade, ointment of mercury, or mezereum bark.

Detection: According to Blum (1916), the eruptions produced may be distinguished from those of the true disease by the fact that they are disseminated and do not form confluent masses. Furthermore, the skin, after the removal of the crust, does not appear red, dry, and hypertrophied, as in true eczema.

HERPES: Certain plants of the family *Euphorbiaceæ*, applied to the skin.

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[1] The presence of the diagnostic signs simulating erythema is not always an indication of fraud. Very often workers in various trades may have raw erythematous appearing hands. As examples of this kind, Collie (1916) gives the following: Hair dressers, through the use of alkaline shampooing liquids; French polishers, through the use of potassium dichromate; carpenters, working with teak or rose wood; tanners, handling arsenic; masons, through the handling of silicates; photographers, through the action of liquids containing chlorine; painters, and those engaged in handling aniline dyes or strong alkalies.